EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Performance Assessment and Sterility Verification of Prepared Media and Reagents

SOP Number: QC-11-02

Date Revised: 07-18-03

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1.0 SCOPE AND APPLICATION:

1.1 This protocol describes quality control practices that will be performed on prepared media to assess the ability of the media to support recovery/growth of the test organisms and to verify media/reagent sterility.

2.0 DEFINITIONS:

2.1 General growth media = Media which support the growth of a broad range of organisms and are used to grow standard cultures. General growth media are non-selective. Examples include nutrient and T-soy media (see ref. 15.5).

2.2 Enriched media = Media which are designed to support the growth of organisms with unusual growth requirements. The special nutrients added to the media will vary dependent upon the particular requirements of the fastidious organism. An example of an enriched medium is T-soy agar supplemented with sheep's blood (commonly called "blood agar") (see ref. 15.5).

2.3 Selective media = Media that permit the growth of one type of bacterium while inhibiting the growth of other types. This facilitates the isolation of a desired species. Examples include Modified Proskauer Beck Medium, Middlebrook 7H9 Broth and Agar, Kirchner's Medium, Mannitol Salt Agar, and Cetrimide Agar (see ref. 15.5).

2.4 Differential media = Media that allow visual differentiation between two or more species of bacteria. Examples include blood agar (differential only), Mannitol Salt Agar (selective and differential), and Cetrimide Agar (selective and differential) (see ref. 15.5).

2.5 CFU = Colony Forming Unit

3.0 HEALTH AND SAFETY:

3.1 All manipulations of the test organism are required to be performed in accordance with biosafety practices stipulated in SOP MB-01, Biosafety in the Laboratory.

4.0 CAUTIONS:

4.1 Performance assessment using *Mycobacterium bovis* (BCG) should not be conducted using the Autoplate 4000 Automated Spiral Plater.

5.0 INTERFERENCES:

5.1 Contamination of pre-sterilized supplies or autoclaved supplies may interfere with the outcome of this evaluation. Sterility checks of pre-sterilized and autoclaved supplies used to evaluate media performance must be performed prior to sterility and performance evaluations of media and reagents (see SOP QC-12, Sterility of Pre-Sterilized and Autoclaved Supplies).

5.2 All media and reagents must be labeled with appropriate control numbers (see SOP QC-09, Control Numbers) for tracking purposes. Incorrect labeling may interfere with the interpretation of results.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable about the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Inoculating loops

7.2 Incubator with temperature reading at the appropriate temperature specified for the test conditions, organism, and media

7.3 Cultures of *Pseudomonas aeruginosa* (ATCC #15442) and *Staphylococcus aureus* (ATCC #6538)

7.4 Cultures of *Mycobacterium bovis* (BCG) from Organon Teknika

7.5 For *B. subtillis* (ATCC #19659), a commercial preparation of spores will be purchased to provide inoculum. One source is Presque Isle Cultures, 3804 West Lake Rd., P.O. Box 8191, Erie PA 16505.

7.6 Autoplate 4000 Automated Spiral Plater (Spiral Biotech)

7.7 Pre-sterilized filtration unit with pore size of 0.45µM such as Nalgene Analytical Filter Units.

8.0 INSTRUMENT OR METHOD CALIBRATION: Not applicable

9.0 SAMPLE HANDLING AND STORAGE: Not applicable

10.0 PROCEDURE AND ANALYSIS:

10.1 Performance Assessment: The performance verification of media used to support official efficacy testing (including the AOAC Use-Dilution Test Method, the AOAC Germicidal Spray Products Test, the Germicidal Towelette Products Test, or the AOAC Confirmatory Tuberculocidal Test) should be done prior to or concurrently with testing.

10.1.1 Culture Preparation: Use testing inoculum prepared for official efficacy testing or from stock cultures, inoculate liquid media and incubate as follows:

For *S. aureus*, *P. aeruginosa*, or *B. subtilis* cultures, inoculate nutrient broth or synthetic broth and incubate at 37±1°C for 24±2 or 51±3 hours.

For *M. bovis* (BCG) inoculate Modified Proskauer Beck medium and incubate at 37±1°C for 21-25 days. Prepare culture with 20% Transmittance (at 650 nm) as described in SOP MB-07, Confirmatory Tuberculocidal Method for Testing Disinfectant Efficacy**.**

10.1.2 For each batch of ***general growth, solid media in plates*** which is used for enumeration or subculturing (*i.e.*, trypticase soy agar), 6-8 petri dishes will be reserved to assess the media's performance. Use a spread plate technique and inoculate plates in duplicate with serial ten-fold dilutions of a test microbe culture that will readily grow on it. For *S. aureus*, *P. aeruginosa*, or *B. subtilis*, use 1 mL of the 1×10^{-6} to 1×10^{-8} dilutions for inoculation.

For ***general growth, solid media in tubes*** (*i.e.*, nutrient agar, cystine trypticase agar), streak or stab inoculate a minimum of two tubes per batch with a suitable undiluted culture.

10.1.3 For each batch of ***selective, solid media in plates*** which is used for enumeration or subculturing (*i.e.*, M7H9 agar), 6-8 petri dishes will be reserved to assess the media's performance. Use a spread plate technique to inoculate plates in duplicate with serial ten-fold dilutions of *M. bovis* (BCG). From a *M. bovis* (BCG) prepared suspension with a 20% Transmittance at 650 nm, use 1 mL of the 1×10^{-5} to 1×10^{-7} dilutions for inoculation.

For ***selective, solid media in tubes*** (*i.e.*, M7H9 slants used for stock transfers), streak inoculate a minimum of two tubes per batch with a suitable culture of *M. bovis* (BCG).

10.1.4 Incubate inoculated media at 37±1°C for 24±2 h for *S. aureus*, *P. aeruginosa*, and *B. subtilis*, and at 37±1°C for 21-25 days for *M. bovis* (BCG).

10.1.5 Results: For ***general growth and selective solid media in plates***, the CFUs are enumerated and the colony morphology is assessed (see Table 1). Record findings on form 16.1, Performance and Sterility Assessment of Media in Plates.

Performance results for media in plates used for enumeration is based on performance data from previous batches of the same media. The ranges specified in 10.1.2 and 10.1.3 should yield plates with CFU counts in the range appropriate for determining the CFU/mL of the starting inoculum (30-300). If CFU counts are below this range, then either the media performance is unsatisfactory or the starting inoculum was substandard. The media performance assessment should be repeated and the final assessment based on an analysis of both sets of data. For M7H9 plates,

it may be necessary to substitute carrier count data (from a test for which the media may have been used) rather than conduct a second performance assessment.

Results: For ***general growth, solid media in tubes*** receiving a streak or stab, the growth and appearance of the test microbe are assessed (see Table 1). Record findings on the Performance and Sterility Assessment of Media in Tubes form (see 16.2). If growth occurs and exhibits typical morphology a "+" will be recorded. If no growth is apparent a "0" will be recorded. For atypical growth that is determined not to be a test microbe, record observations and indicate growth was a contaminant. Complete the form under the "Performance Assessment" caption, by checking either Satisfactory or Unsatisfactory per the observations.

10.1.6 For each batch of ***selective, solid media in plates*** which is not used for enumeration or subculturing (*i.e.*, mannitol salts agar, Cetrimide agar), inoculate by isolation streak with the specific organism for which the media is designed to identify. Incubate cultures at 37±1°C for 24±2 hours for *S. aureus* and *P. aeruginosa*. Following incubation, assess the growth and appearance of the test microbe (see Table 1). If the culture exhibits typical morphology and appearance, a "+" will be recorded on the Performance and Sterility Assessment of Media in Plates form (see 16.3). If no growth is apparent a "0" will be recorded. For growth that is determined not to be a test microbe, record observations and indicate growth was a contaminant. On the form under the "Performance Assessment" caption check either Satisfactory or Unsatisfactory per the observations.

Table 1. Typical Growth Characteristics of *P. aeruginosa, S. aureus, B. subtilis,* and *M. bovis* (BCG)

	*P. aeruginosa**	*S. aureus**	*M. bovis* (BCG)**	*B. subtilis**
Gram rxn.	negative	positive	positive	positive
Acid Fast rxn.	N/A	N/A	Acid Fast	N/A
Typical Growth Characteristics on Solid Media				
Mannitol Salt	N/A	circular, small, yellow colonies, agar turning fluorescent yellow	N/A	N/A
Pseudosel	circular, small, initially opaque, turning fluorescent green over time; agar fluorescent yellowish green	N/A	N/A	N/A
Middlebrook 7H9	N/A	N/A	rough, raised, thick colonies with a nodular or wrinkled surface and an irregular thin margin, off-white to faint buff, or even yellow	N/A
TSA	flat, opaque to off-white, round spreading	small, circular, yellow glistening	N/A	opaque, rough, dull, round, low convex colonies with irregular margins
Typical Microscopic Characteristics				
Cell dimensions	0.5-1.0 μm in diameter by 1.5-5.0 μm in length*	0.5-1.5 μm in diameter*	0.3-0.6 μm in diameter by 1-4 μm in length**	0.8-1.0 μm in diameter by 3.5-5.0 μm in length*
Cell appearance	straight or slightly curved rods, single polar flagella, rods formed in chains	spherical, occurring singly, in pairs and tetrads, sometimes forming irregular clusters	rods, straight or slightly curved, occurring singly and in occasional threads	rods, singly or in pairs, motile by peritrichous flagella, production of central spores

*After 24±2 hours
**After 20±5 days

10.1.7 For ***broth media*** used for propagation, sequential transfers or subculturing of test organisms (*i.e.*, Letheen broth, nutrient broth, Modified Proskauer Beck medium), 6-8 tubes for each batch will be kept aside for the growth assessment test.

10.1.7.1 Inoculate tubes in duplicate with serial ten-fold dilutions of an appropriate test culture. Inoculum should be diluted to a level such that one set (two tubes) is challenged with a reasonably low number of CFUs [in the range of 1-30 CFUs for *S. aureus*, *P. aeruginosa*, and *B. subtilis* and a minimum of 50 CFUs for *M. bovis* (BCG)]. Verify the CFU of the inoculum by plating the ten-fold dilutions used to prepare the inoculum on appropriate media.

For *B. subtilis*, use 1 mL of the appropriate dilutions of a spore suspension (follow guidelines in section 10.2 of SOP MB-12, Sporicidal Neutralization Test, for diluting a concentrated spore suspension that has been sheered from carriers or purchased from a supplier).

For *S. aureus* or *P. aeruginosa*, use 1 mL of the 1×10^{-6} to 1×10^{-8} dilutions for inoculation.

For *M. bovis* (BCG) use 1 mL of the 1×10^{-5} to 1×10^{-7} dilutions for inoculation.

10.1.7.2 Incubate the media at 37±1°C for 24±2 h for *S. aureus*, *P. aeruginosa*, and *B. subtilis* and for *M. bovis* (BCG), incubate the media at 37±1°C for at least 21 days; if no growth is observed at 21 days, incubation may be continued for up to 60 days.

10.1.7.3 Following incubation, the performance of each medium will be visually assessed and the observations will be recorded on form 16.2, Performance and Sterility Assessment of Media in Tubes. Plate counts will be read to determine the number of CFUs delivered to each set of tubes. For each tube, a "+" will be recorded if growth is observed (indicated by turbidity) and a "0" will be recorded if growth is not observed. In the case of a contaminant, record observations and indicate growth was a contaminant.

Performance is judged to be satisfactory when at least one of the tubes in the dilution set receiving the sufficiently low challenge of CFUs (see section 10.1.7.1) shows growth. The number of CFUs delivered to each tube in a set is based on the corresponding averaged plate counts for that dilution. All tubes in dilution sets receiving greater than the sufficiently low challenge of CFUs should show growth as well. Based on this criteria, under the "Performance Assessment" caption on the form, check either Satisfactory or Unsatisfactory. Also record the performance assessment on the Media/Reagent Preparation and Performance Log Form (see SOP MB-10, Media and Reagents).

10.1.8 Use of the Spiral Plater for Performance Assessment: Alternatively, the Autoplate 4000 Spiral Plater may be used for Performance Assessment of ***general growth, solid media in plates***. Dilutions for *S. aureus* and *P. aeruginosa* should be performed as outlined in Section 10.1.2. The Autoplate 4000 Automated Spiral Plater should not be used to assess the performance of *Mycobacterium bovis* (BCG). See SOP QC-20, Spiral Plater, for instructions on use of the Spiral Plater.

10.2 Sterility Verification: The sterility verification of media used to support official efficacy testing (including the AOAC Use-Dilution Test Method, the AOAC Germicidal Spray Products Test, the Germicidal Towelette Products Test, or the AOAC Confirmatory Tuberculocidal Test) should be done prior to or concurrently with testing. Sterility verification must be performed on a minimum of 2% of each batch of solid or liquid media. For those media/reagents that will be used in the AOAC Sporicidal Activity Test, a secondary incubation at 55±1°C will be included.

10.2.1 Plates or tubes of media, for organisms other than *M. bovis* (BCG), may be placed directly in an incubator at 37±1°C and incubated for 5-10 days. Media used for the growth of *M. bovis* (BCG) are incubated at 37±1°C for 21-60 days unless any contamination is observed before then. Following incubation, if no growth on solid media or in broth media is observed, record a "0." If growth is observed, a "+" is recorded on the appropriate Performance and Sterility Assessment form (see 16.0). On each form, fill-in "Sterility Assessment" by indicating either Satisfactory or Unsatisfactory per the observations. Also record the sterility assessment on the Media/Reagent Preparation and Performance Log Form (see SOP MB-10, Media and Reagents).

For media/reagents that will be used in the AOAC Sporicidal Activity Test, the above procedure is repeated with incubation at 55±1°C for 5-10 days. Sterility results should be filled in similarly.

10.2.2 Sterility of reagents that are not intended to support microbial growth:

If a reagent is dispensed in bottles, **each bottle** should be checked for sterility. Use a pre-sterilized 0.45µm filter unit and aseptically filter 2% of the volume of each bottle of reagent. In the BSC, remove the lid from each bottle in a batch and pour 2% of the volume into the pre-filtration reservoir. There are graduations on the reservoir that are sufficiently accurate for this purpose. Do not place anything

such as a pipette into the reagent bottle. Because microbial contamination is expected to be sufficiently low or nonexistent, 2% of a batch may be filtered using the same filter unit. Once filtration is complete, aseptically transfer the filter to a TSA plate and incubate for 5-10 days at 37±1°C.

For reagents that are dispensed in tubes, set aside the number of tubes that represents 2% of the total volume of media prepared. For example, if 1 liter of dilution water is prepared in 9 mL blanks, 2% of 1L would be 20 mL so 3 tubes would be set aside. Using one pre-sterilized filtration unit per batch aseptically transfer the contents of all the tubes to the pre-filtration reservoir. Once filtration is complete, transfer and incubate the filter for 5-10 days at 37±1°C.

Following incubation, if no growth is observed, record a “0" and if growth is observed record a “+” on the appropriate form. In the case of reagents, record the assay results and observations on form 16.3, Sterility Verification for Reagents, as well as on the Media/Reagent Preparation and Performance Log Form (see SOP MB-10, Media and Reagents).

For media that will be used in the AOAC Sporicidal Activity Test, the above procedure is repeated with incubation at 55±1°C for 5-10 days. Sterility results should be filled in similarly. Duplicate sets of tubes, plates, or filters may be prepared and incubated at 55±1°C concurrently with incubation at 37±1°C. Alternately, if no growth was observed following incubation at 37±1°C, the same tubes, plates, or filters, may then be incubated at 55±1°C for an additional 5-10 days.

10.2.3 For solid media in plates or tubes, following incubation, if no growth on solid media or in broth media, a “0" is recorded. If growth is observed, a “+” is recorded on the appropriate Performance and Sterility Assessment form (see 16.0). On

each form, fill-in "Sterility Assessment" by indicating either Satisfactory or Unsatisfactory per the observations. Also record the sterility assessment on the Media/Reagent Preparation and Performance Log Form (see SOP MB-10, Media and Reagents).

For general growth and selective, solid media in plates, satisfactory sterility results indicate that no growth was observed after the incubation period on any uninoculated plates. Unsatisfactory results indicate that some "substantial" form of growth was observed on any of the uninoculated plates resulting from inadequate sterilization or poor aseptic technique when pouring the plates. Colonies observed within the media (substantial growth in this case) are more indicative of inadequately sterilized media than are colonies only on the surface. Surface colonies seen on only one plate (where 2 or more plates were incubated) may indicate contamination of the media subsequent to sterilization and may lead to a false conclusion about the sterility of the remaining batch. Caution and careful interpretation of sterility results must be exercised when interpreting results where only surface colonies are present on only a portion of the plates incubated for sterility assessment. In cases where only surface colonies are seen, a decision on sterility is best made on the basis of the relative abundance of the surface colonies. If only one colony is observed on one plate where two or more were incubated, the media can be assessed as satisfactory or sterility repeated on another set of plates from the same batch if still available. If more than one colony is observed on one plate or one colony is observed on two or more plates, the media is assessed unsatisfactory. In this case, reassess the sterility using twice as many plates as used originally. If any growth is observed, discard the media as unsterile.

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate forms. Completed forms are archived in notebooks kept in locked file cabinets in the file room D217. Only authorized personnel have access to the locked files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The OPP Microbiology Laboratory conforms to 40CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

13.2 For quality control purposes, the required information is documented on the appropriate record form(s) (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Any media/reagent failing (unsatisfactory) a growth assessment or sterility verification will be discarded.

14.2 When the media performance and sterility assessment are performed concurrently with the use of the media in testing and the media quality is deemed unsatisfactory, the specific use of the media in the test(s) should be investigated to determine the potential impact on the test results and the necessity for repeating the test(s). Testing must be repeated when unsatisfactory media and/or reagents are used for critical aspects of testing such as subculturing, confirmation testing, carrier counts and serial dilutions.

14.2.1 If the same preparation of media is available, it should be reevaluated for performance and/or sterility (failed component only) per this SOP. If the preparation is deemed satisfactory, it is not necessary to repeat the test(s).

14.2.2 If the unsatisfactory preparation is not available, investigate other sources of performance data such as test controls and neutralization tests. If additional data indicates satisfactory performance, repeating the test may not be necessary.

14.2.3 Document all findings on the appropriate performance and sterility forms.

15.0 REFERENCES:

15.1 Cunnif, P., ed. 1998. Disinfectants. In: Official Methods of Analysis of AOAC International, 16th Edition, 4th Revision. AOAC International, Gaithersburg, MD.

15.2 Holt, J., Krieg, N., Sneath, P., Staley, J. and Williams, S. eds. 1994. Bergey's Manual of Determinative Bacteriology, 9th Edition. Williams & Wilkins, Baltimore, MD.

15.3 Krieg, Noel R. and Holt, John, G. eds. 1984. Bergey's manual of Systematic Bacteriology Volume 1. Williams & Wilkins, Baltimore, MD.

15.4 Sneath, P., Mair, N., Sharpe, M.E., and Holt, J. eds. 1986. Bergey's Manual of Systematic Bacteriology Volume 2. Williams & Wilkins, Baltimore, MD.

15.5 Brock, T., Madigan, M., Martinko, J., Parker, J. 1994 Biology of Microorganisms, 7th Edition. Prentice-Hall Inc., Englewood Cliffs, NJ.

16.0 FORMS AND DATA SHEETS:

16.1 Performance and Sterility Assessment of Media in Plates

16.2 Performance and Sterility Assessment of Media in Tubes

16.3 Sterility Verification of Reagents

Performance and Sterility Assessment of Media in Plates

OPP Microbiology Laboratory

Background and Preparation	
Date Performed/initials:	
Test organism/control number:	
Media tested/prep number:	
Media used for enumeration:	☐ Yes ☐ No Fill in the appropriate Results block below
Total volume of media prepared:	
Number of plates analyzed for performance:	
Number of plates analyzed for sterility:	

Media Performance and Sterility Results for Plates **USED IN ENUMERATION**						
Performance: Plate Count Data Date/Initials:_____					Sterility Assessment† Date/Initials:_____	
Dilution	Plate 1	Plate 2	Avg. CFU/mL	Observations	Sterility (+/0)	Sterility (+/0)
					Plate# ___=___	Plate# ___=___
					Plate# ___=___	Plate# ___=___
					Plate# ___=___	Plate# ___=___
					Plate# ___=___	Plate# ___=___

Media Performance and Sterility Results for Plates **NOT USED IN ENUMERATION**			
Performance: Plate Count Data Date/Initials:_____		Sterility Assessment† Date/Initials:_____	
Growth (+/-)	Observations	Sterility (+/0)	Sterility (+/0)
Plate# ___=___	Plate# ___=___	Plate# ___=___	Plate# ___=___
Plate# ___=___	Plate# ___=___	Plate# ___=___	Plate# ___=___
Plate# ___=___	Plate# ___=___	Plate# ___=___	Plate# ___=___
Plate# ___=___	Plate# ___=___	Plate# ___=___	Plate# ___=___

† + = growth, 0 = no growth

Performance Assessment Satisfactory ☐ Unsatisfactory ☐

Sterility Assessment @ 37±1°C Satisfactory ☐ Unsatisfactory ☐

Performance and Sterility Assessment of Media in Tubes

OPP Microbiology Laboratory

Background and Preparation	
Date performed/initials:	
Test organism/control number:	
Media tested/prep number:	
Media Type and Incubation Temps:	☐ Solid ☐ Liquid ☐ 37°C ☐ 55°C
Total volume of media prepared:	
Number of tubes analyzed for performance:	
Number of tubes analyzed for sterility:	
Plating media/prep number:	☐ N/A

Results of Media Performance of Liquid Media in Tubes (37°C) Date/Initials:_____						
Plate Counts				Performance Assessment at Each Dilution†		
Dilution	Plate 1	Plate 2	Avg. CFU/mL	Tube 1: (+/0)	Tube 2: (+/0)	Observations

Results of Sterility Assessment of **LIQUID MEDIA IN TUBES**			
37±1°C Results Date/Initials:_____		55±1°C Results Date/Initials:_____	
Growth (+/0)	Growth (+/0)	Growth (+/0)	Growth (+/0)
Tube# ___=___	Tube# ___=___	Tube# ___=___	Tube# ___=___
Tube# ___=___	Tube# ___=___	Tube# ___=___	Tube# ___=___
Tube# ___=___	Tube# ___=___	Tube# ___=___	Tube# ___=___
Tube# ___=___	Tube# ___=___	Tube# ___=___	Tube# ___=___

Sterility and Media Performance Results of **SOLID MEDIA IN TUBES** (37°C)			
Performance† Date/Initials:_____		Sterility Assessment† Date/Initials:_____	
Tube# ___=___	Tube# ___=___	Tube# ___=___	Tube# ___=___
Tube# ___=___	Tube# ___=___	Tube# ___=___	Tube# ___=___
Tube# ___=___	Tube# ___=___	Tube# ___=___	Tube# ___=___
Tube# ___=___	Tube# ___=___	Tube# ___=___	Tube# ___=___

† + = growth, 0 = no growth

Performance Assessment Satisfactory ☐ Unsatisfactory ☐

Sterility Assessment @ 37±1°C Satisfactory ☐ Unsatisfactory ☐

Sterility Assessment @ 55±1°C N/A ☐ Satisfactory ☐ Unsatisfactory ☐

Sterility Verification of Reagents
OPP Microbiology Laboratory

Background and Preparation	
Date performed/initials:	
Reagent tested/prep number:	
Total volume of reagent prepared:	
Batch Volume Distribution Information: Indicate whether reagent was dispensed into bottles or tubes, the total number of bottles or tubes in the batch, the volume of each bottle or tube, and the volume that represents 2% of each bottle, or the number of tubes that represents 2% of the total volume dispensed into tubes.	☐ Bottles\#_____ Volume/bottle_____ Volume analyzed per bottle:_____
	☐ Tubes\#_____ Volume/tube_____ Total number of tubes analyzed:_____
Filter Unit/ Control Number	
Culture (plating) media/prep number:	

Results of Sterility Verification			
37±1°C Results Date/Initials:_____		55±1°C Results Date/Initials:_____	
Incubation start date		Incubation start date	
Incubation stop date		Incubation stop date	
Was growth observed?	☐ Yes ☐ No	Was growth observed?	☐ Yes ☐ No
If Yes, how many colonies?		If Yes, how many colonies?	
Comments/Corrective Action:			

Additional Comments:

Sterility Assessment @ 37±1°C Satisfactory ☐ Unsatisfactory ☐

Sterility Assessment @ 55±1°C N/A ☐ Satisfactory ☐ Unsatisfactory ☐